COMPLEMENT-FIXATION IN GONOCOCCUS INFECTIONS *

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Following the application of the principles of complement-fixation in the serum diagnosis of syphilis, the possibilities of this method as a means of diagnosis were soon realized and in a short space of time many infections were studied. In no instance, however, have results been secured comparable to the diagnostic value of the syphilis reaction. This is probably due to two main factors: (1) syphilis quickly becomes a general infection with a resulting extensive antibody formation, and (2) the antibody of the treponema pallidum or at least the more prominent of two possible antibodies, is characterized by its great affinity for lipoids in tissue extracts (antigens). This peculiar lipodophilic antibody or reagin is found with any degree of constancy in only two other infections, frambesia and leprosy. Pure culture antigens of the treponema pallidum have not the same value in the syphilis reaction as the ordinary lipoidal extracts, and results in the serum diagnosis of syphilis with these culture antigens are comparable in their inconstancy and weakness to reactions in other infectious diseases with their specific culture antigens, including the gonococcus complement-fixation test. If Wassermann and his co-workers had had a pure culture antigen of the treponema pallidum instead of fortunately but unwittingly selecting a tissue the great value of the syphilis reaction would not have been so quickly realized.

In 1906, Muller and Oppenheim[1] applied the complement-fixation test in the diagnosis of gonorrheal arthritis, using a culture of the gonococcus as antigen. To them, therefore, belongs the credit of having recorded the first complement-fixation test in gonococcus infection. A little later in the same year, Carl Bruch,[2] who applied the reaction in 3 cases of gonorrhea and with the serum of immunized rabbits, reported favorable results. In 1907 Meakins[3] reported positive reactions in 3 cases of gonorrheal arthritis and was the first in America to make a report on the subject. Vannod,[4] who studied the specificity of the reac-

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1. *Wien. klin. Wchnschr.*, 1906, 19, p. 894.
2. *Deutsch. Med. Wchnschr.*, 1906, 32, p. 1368.
3. *Johns Hopkins Hosp. Bull.*, 1907, 18, p. 255.
4. *Deutsch. Med. Wchnschr.*, 1906, 32, p. 1984.

tion with sera of rabbits immunized with gonococcus proteid and one of a meningococcus, reported that the meningococcus immune serum did not show complement fixation with gonococcus antigen, and vice versa, that gonococcus amboceptor was not found by meningococcus antigen. Wollstein,[5] in a study of the biologic relationship of the gonococcus and meningococcus, reported results differing from those of Vannod. She found the bacteriolytic amboceptors in the sera of rabbits, immunized with these cultures, to be closely related and yielding fixation of complement with either antigen. Teague and Torrey[6] followed with a very important communication showing that the differences in results of previous investigators were probably due in part to the use of single strains of the organisms in the preparation of antigens and immune sera. They emphasized the fact that the gonococcus belongs to a heterogeneous family and in attempting the diagnosis of gonorrheal infections by the complement-fixation method extracts of several different strains should be used. Naz Vannod,[7] and later Watabiki,[8] found that the gonococcus and meningococcus antibodies were quite specific for the homologous antigens in complement-fixation reactions.

Particular attention was drawn to the gonococcus complement-fixation test by the work of Schwartz and McNeal.[9] These investigators emphasized the necessity of using polyvalent antigens, and their encouraging reports have stimulated renewed interest in this subject. They found that a positive reaction was not obtained if the infection was confined to the anterior urethra, and that a strong reaction was not to be expected before the fourth week of the infection and then only in acute cases with complications. They regard a positive reaction as indicating the presence, or rather activity in the body, of a focus of living gonococci, although a negative reaction does not exclude gonococcus infection. A positive reaction was secured in 31.4 per cent. of cases regarded clinically as gonorrhea; in 54.8 per cent. of cases of chronic prostatitis giving a positive history of infection within three years; in 13.2 per cent. of cases regarded as clinically cured for at least three months. They secured positive reactions in a certain number of cases, especially in women, when the bacteriological examination failed to show gonococci. The test therefore has a more positive than negative value. With Flexner's antimeningococcus serum positive reactions resulted with their gonococcus antigen—with sera from cases of cerebrospinal meningitis (meningococcus) the results were negative.

In the following years numerous reports, including those of Swinburne,[10] Gradwohl,[11] O'Neil,[12] Gardner and Clowes,[13] have emphasized the practical value of the gonococcus complement-fixation test, particularly as an aid in determining whether or not a patient is cured.

Since the quantity of antibody formed in a strictly local gonococcus infection is probably small because of the comparatively slight cellular involvement, the complement-fixation reactions are generally weak and consequently require the closest technical attention especially in the preparation of antigen and in the accurate adjustment

5. *Jour. Exper. Med.*, 1907, 9, p. 588.
6. *Jour. Med. Research*, 1907, 17, p. 223.
7. *Centralbl. f. Bakteriol.*, 1907, 44, p. 10.
8. *Jour. Infect. Dis.*, 1910, 7, p. 159.
9. *Am. Jour. Med. Sc.*, 1911, 143, p. 693; *ibid.*, 1912, pp. 144, 369 and 815.
10. *Arch. Diagnosis*, 1911, 4, 3, p. 227.
11. *Am. Jour. Dermat. and Syph.*, 1912, 16, p. 294.
12. *Boston Med. and Surg. Jour.*, 1912, 167, p. 464.
13. *New York Med. Jour.*, 1912, 96, p. 734.

of the hemolytic system. There can be no doubt of the truth of the contentions of Teague and Torrey, Schwartz and McNeil, that the more polyvalent the antigen the more satisfactory the complement-fixation tests. One of our interests in this subject was centered on methods of preparing antigens, and whether best results are secured by using an antigen composed essentially of the endotoxins of gonococci, or of the bacterial protein, or both.

The question of the rôle of secondary infection in chronic gonococcus infections of the genito-urinary tract is one of much interest. One of us having isolated a large number of cultures of staphylococci, streptococci, diphtheroid bacilli, etc., from cases of chronic urethritis and prostatitis, prepared antigens of these organisms and used them with all sera in an endeavor to determine their etiological relationship to these chronic processes, in so far as this could be done by complement-fixation reactions with homologous and mixed antigens.

Since the gonococcus, meningococcus, and the micrococcus catarrhalis possess morphological and biological characters in common, a study of their biological relationship by complement-fixation reactions with their various immune sera and antigens is of distinct importance as it has a direct bearing on the specificity of the gonococcus fixation test.

The objects of our study were mainly four-fold: (1) To ascertain the practical value of gonococcus antigen prepared after various methods; (2) to study the relation of mixed infection to chronic gonococcus infections of the genito-urinary tract by complement-fixation experiments with antigens of the organisms commonly found in mixed infections; (3) to study the practical value of the gonococcus fixation test in so far as it could be done with the material used in our investigations; (4) to study the biological relationship of the gonococcus to the meningococcus and the micrococcus catarrhalis by means of complement-fixation tests.

MATERIALS

Sera.—Of the 92 sera used, 73 were of persons giving positive bacteriological evidence of a clear history and definite clinical evidence of gonococcus infection, and nineteen were of persons giving a negative history and presenting no clinical evidences of infection. As gonorrhea is so widespread it was impossible to determine how many of these had had an infection at a remote and forgotten date. Five of these sera, however, were obtained from young boys in whom infection could be excluded with certainty.

Cultures.—The following cultures were used in preparing antigens: Eight strains of gonococci, all of which were originally secured from the Research Laboratory of the New York Board of Health; six strains of meningococci, five of which were secured from the Research Laboratory of the New York Board of Health, while the sixth was isolated from a case of meningitis by one of us; six strains of micrococcus catarrhalis, which were isolated from sputum and excised tonsils; three strains of staphylococcus albus and three of staphylococcus aureus, isolated from cases of chronic prostatitis; six strains of streptococci, isolated from cases of chronic prostatitis, and six strains of diphtheroid bacilli, isolated from cases of chronic prostatitis.

Antigens.—These were prepared as follows:

1. The washed gonococci were suspended in normal saline solution (unheated). Eight strains of gonococci were grown on serum agar for 4 days at 38 C. and growths washed off with sterile saline solution and centrifuged. The supernatant fluid was discarded and the sediment mixed with an excess of saline and centrifuged. The supernatant fluid was discarded and the sediment suspended in fresh sterile saline solution. To this suspension was added one-half of 1 per cent. phenol.

2. The washed gonococci were suspended in normal saline solution (heated). Eight strains were grown on blood agar, washed and suspended in Antigen 1. The emulsion was then heated at 60 C. for one hour and a preservative added.

3. Gonococcus antigen, purchased in the open market, was prepared after the following method:

Twenty-hour cultures on ascites agar in large flat quart flasks were washed off with physiological salt solution, shaken for eighteen hours, 0.2 per cent. trikresol added, and then passed through a Berkefeld filter. It was then diluted so that each 28 square inches of surface growth gave 16 c.c. of the antigen. This antigen was made of fourteen strains of the micrococcus gonorrhea.

4. Gonococcus antigen was prepared after the method of Besredka as modified by Gay: The cultures were grown on blood agar for 3 days, washed off with small amounts of saline solution, emulsion centrifuged, and sediment washed 3 times. Sediment was then suspended in sterile saline solution and treated with an equal part of absolute alcohol. After the precipitate had settled the supernatant fluid was drawn off and the sediment dried over sulphuric acid, accurately weighed, ground with crystals of sodium chlorid and made into a 2 per cent. suspension in normal saline solution. This stock dilution was further diluted as needed so that the actual amounts of dry antigenic substance contained in 1 c.c. were as follows:

1/40	dilution	= 0.5	mg.	
1/80	"	" 0.25	"	
1/160	"	" 0.125	"	
1/320	"	" 0.062	"	
1/640	"	" 0.031	"	
1/1280	"	" 0.0155	"	etc.

5. Alcoholic extract of washed gonococci: Seventy-two hour cultures were suspended in normal saline solution, centrifuged, and washed 3 times. The sediment was then treated with 95 per cent. alcohol, incubated at 38 C. for 3 days, shaken mechanically for 3 days more, and then passed through a Berkefeld filter.

The following antigens were prepared by growing cultures of the respective organisms on a serum media for 3 to 5 days, washing off the growths with sterile normal saline solution, centrifuging and washing the sediment 3 times,

suspending the washed sediment in normal saline solution, heating the emulsion to 60 C. for an hour, and adding trikresol as a preservative.

6. Streptococcus (6 strains).
7. Staph. albus (3 strains).
8. Staph. aureus (3 strains).
9. Pseudo-diphtheria bacilli (6 strains).
10. M. catarrhalis (6 strains).
11. Meningococcus (6 strains).
12. Mixed antigen composed of equal parts of Antigens 1-2-4-6-7-8-9-10 and 11.

Filtrates of Emulsions 2-6-7-8-9-10-11 and 12 were prepared as follows, and used as antigens: 5 to 25 c.c. of the antigens, depending on their density, were diluted with sufficient sterile saline solution to bring the total volume to 200 c.c. The diluted emulsions were then shaken mechanically for 72 hours and filtered through sterile Berkefeld filters.

No.	2	a.	Filtrate	of	gonococcus emulsion.
"	4	"	"	"	" "
"	6	"	"	"	streptococcus "
"	7	"	"	"	Staph. albus "
"	8	"	"	"	Staph. aureus "
"	9	"	"	"	B. pseudo-diphteria "
"	10	"	"	"	M. catarrhalis "
"	11	"	"	"	meningococcus "
"	12	"	"	"	mixed emulsions.

TECHNIC

(A) *Hemolytic System*: Both antisheep and antihuman hemolytic systems were employed, the latter as a control over the former to determine the influence of natural antisheep hemolysis in the human sera on the delicacy of the reactions.

With the antisheep system we used the same amounts as in our Wassermann technic, namely, one-half the amounts used in the original Wassermann method. Washed sheep's corpuscles were made up in a 2.5 per cent. suspension and used in doses of 1 c.c.; the fresh sera of guinea-pigs were diluted 1:20 and used as complement in dose of 1 c.c.; the hemolysin was titrated each day, and one hemolytic dose used.

All sera were heated to 55 C. for one-half hour and used in amounts of 0.5 to 0.2 c.c. Serum antigen and complement were incubated for one hour, amboceptor and corpuscles added, incubated for an hour or longer, depending on the hemolysis of the controls, and placed in the refrigerator over night. Readings were made next morning.

By titrating the hemolysin with each complement and corpuscle suspension the hemolytic system was accurately adjusted. The use of a larger amount than is usual in the technic of others is a matter of personal preference, sufficient amount of serum being easily obtained and requiring practically no more antigen. The controls were the same as we use in the routine Wassermann reaction: a serum control with each serum in maximum dosage; antigen, complement, hemolytic and corpuscle controls.

(B) *Antigen Titrations*: Two methods were used, (1) determining the anticomplementary dose of each antigen and using an arbitrary portion of this amount for the antigenic dose, and (2) titration with an antigonococcus immune serum. Of the two methods the former was found more satisfactory because the quan-

tity of gonococcus amboceptor in different immune sera is variable and of no great value in determining the proper dose for human sera, which may contain much less amboceptor.

The anticomplementary dose is easily determined at frequent intervals and if one-quarter or one-half of this amount is used in conducting tests with proper controls there is no danger of false reactions. Furthermore, this was the most feasible method in testing for an unknown amboceptor, as we were testing for staphylococcus, streptococcus, etc., amboceptor in the human sera.

The anticomplementary titrations of our antigens were conducted many times, the doses remaining constant throughout.

Antigen 4 became slightly anticomplementary in dose of 1 c.c. of dilution 1:320 (0.062 mg.). In many of the tests higher dilutions were used with 0.1 to 0.2 c.c. of serum, but with most sera we used but one dose of this antigen, 1 c.c. of dilution 1:1280 (.0155 mg.).

We adopted as our antigenic dose, one-quarter the amount shown by titrations to be anticomplementary. With this amount false reactions were avoided.

RESULTS WITH THE VARIOUS GONOCOCCUS AND OTHER ANTIGENS

The cases have been divided into four main groups: (1) gonorrheal urethritis of males; (2) probable gonococcus infection of women, many of whom came to operation; (3) acute vaginitis in young children; (4) control cases. All of these were submitted from time to time with other sera, the histories of most cases being obtained at a later date.

I. COMPARATIVE VALUES OF VARIOUS GONOCOCCUS ANTIGENS

As shown in the tables, about 60 per cent. of all cases reacted positively with one or more of the gonococcus antigens.

Of particular interest are the comparative results with Antigens 1 and 3. The first, Antigen 1, is a simple suspension of gonococci in normal saline solution and Antigen 3, a filtrate of gonococci autolyzed in normal saline solution. Antigen 1 yielded 58.9 per cent. positive reactions, while Antigen 3 yielded 47.9 per cent. In 51.1 per cent. of cases the reactions with Antigen 1 were stronger than those with Antigen 3, in 39.5 per cent. the reactions were equal in both, and in 9.3 per cent. Antigen 1 yielded weaker reactions.

Antigen 4 yielded but 18.7 per cent. positive reactions. We used this antigen in an amount equaling one-fourth its anticomplementary dose, with 0.1 to 0.2 c.c. of serum.

Antigen 5, an alcoholic extract of gonococci, yielded but 9.3 per cent. positive reactions, and in all of these the reactions were weak. This supports the general observation that alcohol does not serve to extract the antigenic principle of bacteria and is unsuitable for bac-

terial antigens, however desirable it may be from the standpoint of stability.

The filtrates of gonococcus Antigens 2 and 4 were used in testing a number of sera and yielded a much smaller percentage of positive reactions. These antigens were difficult to titrate because of a tendency to become hemolytic. The degree of complement-fixation in positive reactions was always slight, and the readings were difficult.

It is seen that best results were secured with a simple antigen composed of gonococci suspended in sterile normal saline solution plus a preservative. Even in normal saline solution, autolysis rapidly occurs but it appears that the bacterial protein, aside from the endotoxins, possesses antigenic principles and they add to the antigenic value of the preparation. Of great importance in the preparation of gonococcus antigen is the use of as many different strains as possible. The difficulty of isolating cultures of gonococci and keeping them under continuous cultivation over a long period of time and the ever present chances of having the cultures contaminated are factors adding greatly to technical difficulties in the preparation of gonococcus antigens.

II. MIXED INFECTION IN CHRONIC GONOCOCCUS INFECTIONS

All of the cultures of streptococci, staphylococci and pseudodiphtheria bacillus were isolated from cases of chronic urethritis and prostatitis.

By preparing antigens of these organisms, an effort was made to determine their activity in these chronic processes according to whether or not bacteriolytic amboceptors were present in the sera of such cases, in so far as these could be demonstrated by complement-fixation experiments.

In seven, or 9.6 per cent. of cases the antigen of streptococci yielded positive reactions. Four of these were cases of chronic urethritis and prostatitis and three were well marked cases of pyosalpingitis.

In five, or 6.8 per cent. of cases the antigen of white staphylococci yielded positive reactions. One of these was a case of chronic urethritis and prostatitis and four were of pyosalpingitis.

In eight cases, or 11 per cent. the antigen of cultures of staphylococcus aureus yielded positive reactions. Four of these were cases of chronic urethritis and four of pyosalpingitis.

TABLE 1

ANTICOMPLEMENTARY TITRATION OF ANTIGENS

Dose of Antigen	Gonoc. 1 1:50	Gonoc. 2 1:200	Gonoc. 3 1:20	Alcoh. Ext. Gonoc. 1:50	Streptoc. 1:200	S. Albus 1:200	S. Aureus 1:200	Pseudod. B. 1:200	M. Catar-rhalis 1:200	Mening. C. 1:200	Mixed 1:200
0.4 c.c.	H.	H.	H.	H.	H.	H.	H.	H.	H.	H.	H.
0.8 c.c.	S. I. H.	H.	M. I. H.	H.	H.	H.	H.	H.	H.	H.	H.
1.0 c.c.	M. I. H.	H.	I. H.	H.	S. I. H.	H.	H.	H.	H.	H.	H.
2.0 c.c.	I. H.	S. I. H.	I. H.	S. I. H.	M. I. H.	S. I. H.	S. I. H.	S. I. H.	S. I. H.	S. I. H.	S. I. H.
Antigenic dose used	0.2 c.c.	0.5 c.c.	0.2 c.c.	0.5 c.c.	0.4 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.

H=Complete hemolysis.
S. I. H.=Slight inhibition of hemolysis.
M. I. H.=Marked inhibition of hemolysis.
I. H.=Complete inhibition of hemolysis.

TABLE 2.

COMPLEMENT-FIXATION IN GONOCOCCUS INFECTION OF MALES.

No.	Gonoc. 1	Gonoc. 2	Gonoc. 3	Gonoc. 4	Alcoh. Ext. Gonoc.	Streptoc.	S. Albus	S. Aureus	Pseudod. b.	M. Catarrhalis	Meningoc.	Mixed	Diagnosis
1	+++	++	++	0	0	0	0	0	0	0	0	0	Chronic urethritis fourteen years
2	—	—	—	—	—	—	—	—	—	—	—	—	Arthritis; no evidence of urethritis. Denies infection.
3	—	—	—	0	0	0	0	0	0	0	0	0	Chronic urethritis. Several years. No discharge for three months.
9	+	+	+	—	—	—	—	—	—	—	—	—	Urethritis. Three weeks. Arthritis.
10	++	±	+++	—	—	—	—	—	—	—	—	—	Gonorrheal arthritis.
11	—	—	—	—	0	—	—	—	—	—	—	—	Acute urethritis. One week.
12	+	—	+	—	—	—	—	—	—	—	—	—	Urethritis. Sixteen days.
16	—	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis.
17	+	±	+++	—	0	—	0	0	0	—	±	0	Chronic urethritis. Three years.
18	±	+	±	—	—	—	—	0	—	—	0	0	Urethritis. Seven weeks.
19	±	+	±	±	0	—	—	0	0	+	±	0	Urethritis. Six weeks.
20	±	++	—	—	—	+	—	—	—	±	±	—	Urethritis. Five weeks.
21	+++	++	++	±	—	—	—	0	0	+	±	+	Chronic urethritis and prostatitis.
22	±	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis. Ten years.
23	—	—	—	—	—	—	—	0	0	—	—	0	Chronic urethritis and prostatitis. Seven years.
27	+++	±	+	±	0	—	—	±	—	—	—	±	Chronic urethritis.
28	++++	±±	++++	—	0	0	0	—	±	±	—	—	Urethritis. Third attack.
29	++	—	±	±	—	—	—	—	—	—	—	—	Urethritis. Second attack.
30	±	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis.
31	+	+	+	±	—	±	—	—	+	—	++	—	Chronic urethritis. Seventeen years.
32	++++	++++	++	++	0	+	—	±	—	—	++	±	Chronic urethritis and prostatitis.
42	—	±	±	—	—	—	—	—	—	—	—	±	Urethritis. Five months.
43	+	±	±	—	0	—	—	—	—	—	—	—	Chronic urethritis. Four years.
44	+	—	—	—	0	—	—	—	—	—	—	—	Chronic gonococcus epididymitis.
45	—	—	—	—	0	—	—	—	—	—	—	—	Chronic urethritis and prostatitis.

PLATE 2

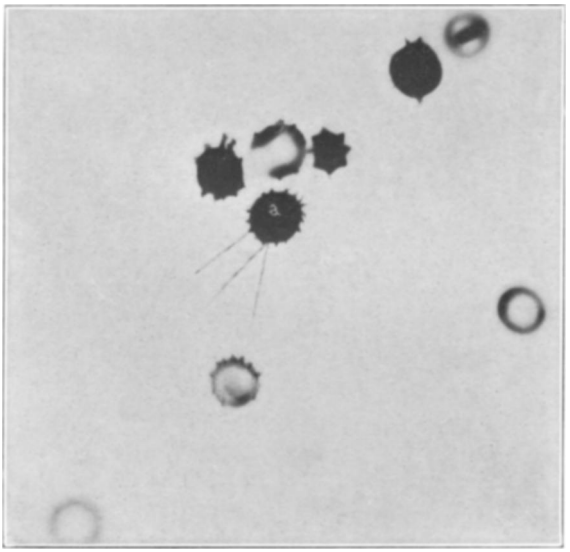

Figure 1

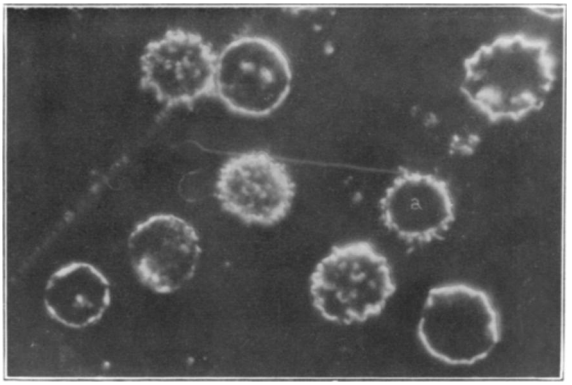

Figure 2

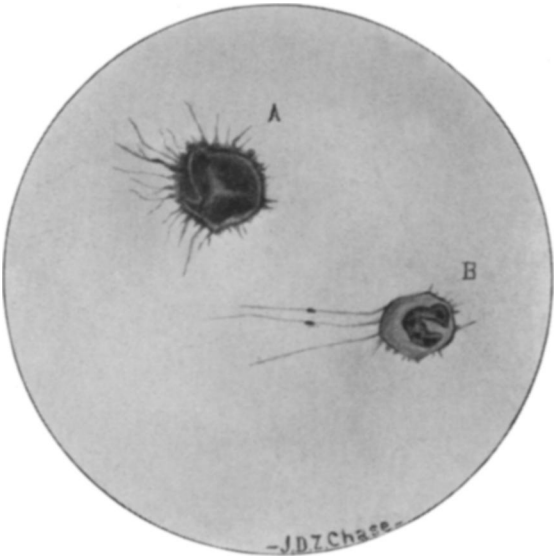

Figure 3

46	—	—	—	—	0	—	—	—	—	—	—	—	Chronic urethritis. One year.
47	+	±	+++	—	0	—	—	—	—	—	—	—	Gonorrheal arthritis.
48	±	+	±	+	—	—	—	—	—	—	—	—	Gonorrheal arthritis.
49	±	±	+	—	0	—	—	—	—	—	—	—	Acute urethritis. Two weeks.
52	—	—	—	—	0	0	—	—	—	—	—	—	Acute urethritis. Six days.
53	—	—	—	—	0	0	0	—	—	—	—	—	Acute urethritis. Ten days.
54	—	—	—	—	0	0	0	—	—	—	—	—	Chronic urethritis. One and one-half years.
55	—	—	—	—	0	0	0	0	0	—	—	—	Chronic urethritis. Two years.
56	—	—	—	—	0	—	—	—	—	—	—	—	Acute urethritis. Three weeks.
57	—	—	—	0	0	0	0	—	—	—	—	—	Acute urethritis. Three and one-half weeks.
58	—	—	—	—	0	—	—	0	—	—	—	—	Acute urethritis. Four weeks.
66	—	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis. One and one-half years.
67	—	—	—	—	—	—	—	—	—	—	—	—	Gonorrheal epididymitis.
69	++	+	++	—	+	+	+	+	—	—	+	+	Chronic urethritis and prostatitis.
70	—	—	—	0	0	0	0	0	0	0	0	0	Urethritis. Five years ago.
71	—	—	—	—	—	—	—	—	—	—	—	—	Urethritis. Six weeks.
72	±	—	±	0	0	—	—	—	—	—	—	—	Urethritis. Ten weeks.
73	++++	++	+++	+	±	—	—	—	—	—	—	—	Urethritis. Eight days.
75	++	+	+	0	0	0	0	—	—	—	—	—	Urethritis. Four weeks.
76	++++	—	—	—	—	—	—	—	—	—	—	—	Urethritis. Four weeks.
77	+	—	—	0	0	0	—	—	—	—	—	0	Urethritis. Fifteen weeks.
78	±	±	—	0	—	—	—	—	—	—	—	—	Urethritis. Sixteen months.
79	++++	++	+	0	0	—	—	±	—	—	—	+	Urethritis. Seven months.
80	—	—	—	—	0	—	—	—	—	—	—	—	Acute urethritis. One week.
83	±	±	±	±	0	—	—	—	—	—	—	—	Chronic urethritis. Three months.
84	+	+	±	—	—	—	—	—	—	—	—	—	Chronic urethritis. Six months.
85	—	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis. Second attack.
86	+++	++	+	—	—	—	—	—	—	—	—	—	Syphilis and gonorrhea.
87	—	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis.
90	±	±	—	—	—	—	—	—	—	—	—	—	Chronic urethritis. Nineteen months.
91	—	—	—	—	—	—	—	—	—	—	—	—	Chronic urethritis. Three months.
92	+	—	+	—	—	—	—	—	—	—	—	—	Gonorrheal arthritis.

++++ Total inhibition (100 per cent.) of hemolysis.
+++ Seventy-five per cent inhibition.
++ Fifty per cent inhibition.
+ Twenty-five per cent inhibition.
± Less than 25 per cent inhibition.
— Complete hemolysis.
0 Not tested.

TABLE 3

COMPLEMENT-FIXATION IN GONOCOCCUS INFECTION OF FEMALES.

	Gonoc. 1	Gonoc. 2	Gonoc. 3	Gonoc. 4	Alcoh. Ext. Gonoc.	Streptoc.	S. Albus	S. Aureus	Pseudod. b.	M. Catarrhalis	Meningoc.	Mixed	Diagnosis
13	+++	±+	+++	±+	0	±+	±+	+±	±+	±+	±+	±+	Bilateral pyosalpingitis.
14	+		+		0								Bilateral pyosalpingitis.
15					0								Abortion case. Indefinite history.
25					0								Indefinite history. Probably lues.
26	++	++	++	+	0	+	++	++	+	0	+	++	Bilateral pyosalpingitis.
68	++		++		±								Salpingitis and lues.

TABLE 4

COMPLEMENT-FIXATION IN VAGINITIS OF CHILDREN.

No.	Gonoc. 1	Gonoc. 2	Gonoc. 3	Gonoc. 4 No.	Alcoh. Ext. Gonoc.	Streptoc.	S. Albus	S. Aureus	Pseudod. b.	M. Catarrhalis	Meningoc.	Mixed	Diagnosis
4	—	—	—	—	0	—	—	—	—	—	—	—	Acute gonorrheal vaginitis. One month.
5	++++	+	++	—	0	—	—	—	—	—	—	—	Scanty discharge. Negative vaginal and vulvar smears. Slight vaginitis.
6	++	+	++	—	—	—	—	—	—	—	—	—	Acute gonorrheal vaginitis. One month. Pus about cervix.
7	—	—	—	—	0	—	—	—	—	—	—	—	Acute vaginitis. No discharge at present.
8	—	—	—	—	—	—	—	—	—	—	—	—	Slight vaginitis. No gonococci at present.
33	++++	+	++	—	0	—	—	—	—	—	—	—	Gonorrheal vaginitis and ophthalmia. Pus about cervix.
34	++	—	±	—	—	—	—	—	—	—	—	—	Acute gonorrheal vaginitis of five weeks' duration.
35	—	—	—	—	0	—	—	—	—	—	—	—	Slight vaginal discharge. No gonococci in discharge.
36	—	—	—	—	0	—	—	—	—	—	—	—	Acute gonorrheal vaginitis of one month. Pus about cervix.
37	+	—	—	—	—	—	—	—	—	—	—	—	Acute gonorrheal vaginitis. Six weeks. Free pus about cervix.

*For more particulars relative to the clinical aspect of these cases see paper of Dr. John F. Sinclair. Archiv. of Pediatrics, December, 1913.

Five, or 6.8 per cent. of cases yielded positive reactions with the antigen of cultures of a pseudo-diphtheria bacillus. Two of these were cases of chronic urethritis and prostatitis and three of pyosalpingitis.

The filtrates of these antigens, prepared after the method already given, presented the same hemolytic properties as noted with the filtrates of the gonococcus antigens. These filtrate antigens were likewise used with a number of sera but the percentage of positive reactions was much less, and the degree of complement-fixation so slight as to make accurate readings quite difficult.

Thus it was found that in about 9 per cent. of cases, all chronic infections, these mixed organisms were insufficiently active to cause the production of amboceptors. This would indicate their importance as secondary factors in these infections and that they were not leading merely a saprophytic existence. This was found especially true in the cases of pyosalpingitis.

III. PRACTICAL VALUE OF THE GONOCOCCUS COMPLEMENT-FIXATION REACTION

The difficulty of isolating and preserving a sufficient number of cultures of real gonococci in order to prepare a satisfactory polyvalent antigen constitutes a weighty drawback in the practical use of the gonococcus complement-fixation test.

In the majority of reactions the amount of complement-fixation was much less than that commonly occurring in syphilis. Hence the reactions were not so satisfactory as those secured in the majority of syphilis reactions, and the readings were more difficult.

Because of the small amount of gonococcus antibody present in the majority of cases, unless accompanied by unusual complications, the technic of the test and particularly the adjustment of the hemolytic system, required considerable care in order to secure best results.

In a comparative study of a number of sera tested with both the antisheep and antihuman hemolytic systems slightly better results were secured with the latter. In using the antihuman system exactly the same technic was employed as with the antisheep system except that two hemolytic doses of hemolysin were used instead of one. In both, inactivated sera were used. The stronger reactions with the antihuman system showed the probable influence of natural antisheep hemolysin in human sera, when an antisheep system is employed.

In 52.6 per cent. of cases the results were the same with both systems; in three cases, or 15.6 per cent. the reactions were slightly positive and one other was stronger with the antihuman and negative or weaker with the antisheep system; in one case the reaction was negative, and in three others weaker with the antihuman, and positive or stronger with the antisheep system. Thus with the antihuman system slightly better results were secured. This was further supported by a similar set of comparative reactions with sera from which the natural antisheep hemolysin had been removed.

The following table gives a summary of the reactions with the ninety-two sera examined:

TABLE 5.

SUMMARY OF COMPLEMENT-FIXATION REACTIONS IN GONOCOCCUS INFECTIONS.

Diagnosis	Reactions		Per Cent. Positive
	+	—	
Urethritis: Week 1-4	6	7	46.1
Week 4-8	3	2	60.0
Week 8-12	3	2	60.0
Over one year	19	10	65.5
Arthritis of probable gonococcus infection	5	1	83.3
Vaginitis (children)	5	5	50.0
Chr. salpingitis	4	2	66.6
Controls: Normal	0	7	0
Lues	1	8	11.1
Chancroids	0	2	0

(a) Positive reactions in uncomplicated urethritis were uncommon under the fourth week of the infection.

(b) As complications developed and more of the body cells were activated to the production of antibodies, the percentage of positive reactions became greater.

(c) The reaction is of particular value in aiding the diagnosis of the nature of an obscure arthritis; in pelvic inflammatory diseases of women; in the examination of women attendants of children; in the determination of whether or not a given case of urethral infection is cured, or still harbors foci of living gonococci; and in the diagnosis and management of gonorrheal vaginitis of female children. Our percentage of positive reactions in such cases of vaginitis would indicate that the infection ascends higher than the cervix more frequently than is generally supposed.

IV. BIOLOGICAL RELATIONSHIP OF THE GONOCOCCUS

The determination of the biological relationship by complement-fixation tests of a micro-organism to others of a group bearing similar morphological or cultural characteristics depends on whether or not the organism produces a specific amboceptor, and the antigen in the test possesses the distinct antigenic principle.

Immune sera, as antimeningococcus sera, differ widely in their amboceptor content as measured by a complement-fixation technic, and hence not all sera are suitable for such study.

Of main interest in this connection is the possible biological relationship of the gonococcus and meningococcus. From the standpoint of similarity in morphology, the micrococcus catarrhalis is also of interest in this connection.

We have studied these relationships by two main methods:

(a) By interactions of the immune sera of these micro-organisms with the respective antigens, and (b) by testing all sera of gonococcus patients with polyvalent antigens of meningococcus and the micrococcus catarrhalis. The results may be summarized as follows:

1. The gonococcus amboceptor fixed complement best with its own antigen, to a less degree with meningococcus antigen, and not at all with an antigen of catarrhalis.

TABLE 6.

ANTIGONOCOCCUS SERUM.

Dose of Serum	Antigens				
	Gon. 1 0.2 c.c.	Gon. 2 0.5 c.c.	Gon. 3 0.2 c.c.	M. Cat. 0.5 c.c.	Meningo. 0.5 c.c.
.01 c.c.	H.	H.	H.	H.	H.
.05 c.c.	S. I. H.	H.	H.	H.	H.
.1 c.c.	S. I. H.	H.	S. I. H.	H.	H.
.2 c.c.	M. I. H.	S. I. H.	M. I. H.	H.	S. I. H.
.2 c.c.	H.	Control			

TABLE 7.

ANTIMENINGOCOCCUS SERUM.

Dose of Serum	Antigens				
	Gon. 1 0.2 c.c.	Gon. 2 0.5 c.c.	Gon. 3 0.2 c.c.	M. Cat. 0.5 c.c.	Meningo. 0.5 c.c.
.01 c.c.	H.	H.	S. I. H.	H.	H.
.05 c.c.	H.	H.	H.	H.	H.
.1 c.c.	S. I. H.	S. I. H.	S. I. H.	H.	H.
.2 c.c.	S. I. H.	S. I. H.	M. I. H.	H.	S. I. H.
.2 c.c.	H.	Control			

The sera used in the experiments illustrated in Tables 6 and 7 were obtained in the open market. Sera of other make obtained in the same way gave similar results.

2. The meningococcus amboceptor in both sera of different sources, while fixing complement to but a slight degree with meningococcus antigen, reacted equally well with gonococcus antigens; no fixation occurred with catarrhalis antigen.

3. These results indicate the close biological relationship of the gonococcus and meningococcus and while their respective amboceptors were most specific for their own antigen, yet in lower dilutions of serum this specificity was lost and the results constituted an example of "group" reaction as seen in similar studies with the group of streptococci, diphtheria bacilli, etc.

4. As shown in Tables 2, 3 and 4, complement-fixation with sera of gonococcus infections reacted positively with the antigen of meningococcus in 13.8 per cent. of cases. This may be considered further evidence of the close relationship of the amboceptor of these organisms, rather than an indication of the presence of a separate meningococcus antibody.

SUMMARY

About 60 per cent. of all cases of gonococcus infections reacted positively in the gonoccocus complement-fixation test. In the few cases of pyosalpingitis examined, 66 per cent. reacted positively. The highest percentage of positive reactions, 83 per cent., occurred in cases of arthritis, considered clinically as possible gonococcus infections.

The gonococcus complement-fixation test is of particular value in aiding the diagnosis of the nature of an obscure arthritis, in pelvic inflammatory diseases of women, to deciding whether or not a given case of urethral infection is cured or still harbors foci of living gonococci and aiding in the diagnosis and management of vaginitis in female children.

The reactions are not generally as satisfactory as those occurring in the syphilis reaction because the quantity of gonococcus antibody is much smaller unless grave and widespread gonococcus metastases exist, and the fixation of complement by bacterial amboceptor and antigen is not so marked as that occurring with syphilis reagin and a lipoidal extract.

In a comparative study of a number of sera tested with both the antisheep and antihuman hemolytic systems slightly better results were secured with the latter.

To be of any value gonococcus antigens must be polyvalent. An antigen composed of a simple suspension of organisms in saline solu-

tion yielded 11 per cent. better reactions than filtrates. It appears that the bacterial protein, aside from the endotoxins, aids in the antigenic effect. Alcoholic extracts of gonococci possess little or no value.

The occurrence of positive reactions in about 9 per cent. of cases of chronic gonococcus infections with antigens of staphylococci, streptococci and diphtheroid bacilli, indicates the active rôle these organisms may assume in these infections. The occurrence of about 5 per cent. positive reactions with an antigen of the micrococcus catarrhalis would indicate that this organism may be likewise active in chronic urethritis.

A study of antigonococcus and antimeningococcus sera with antigens of gonoccoci and meningococci, indicates the close biological relationship of the gonococcus and meningoccocus, and while their respective amboceptors are most specific for their own antigens, in lower dilutions this specificity is not so apparent and the results constitute another example of "group" reaction similar to those occurring with the group of streptococci, diphtheria bacilli, spirochetes, etc.

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